Leukaemia Diagnosis Interpretation
All Participants

Participant: 42981 **Trial No:** 151601

Date Issued: 06-July-2015 **Closing Date:** 24-July-2015

Trial Comments

Your Diagnosis: No Diagnosis

Consensus Diagnosis: T-cell prolymphocytic leukaemia

Your Diagnosis is classed as a **NON RETURN** result

Performance Score: 50

Running Score: 100

Consensus

	Consensus Diagnosis	Your Diagnosis
Lineage	Lymphoid T cell	No Diagnosis
Subclassification	Mature T-cell and NK-cell neoplasms	No Diagnosis
Diagnosis	T-cell prolymphocytic leukaemia	No Diagnosis

Breakdown of returned diagnoses

Diagnosis	No Of Returns	Percentage Of Returns
Total Returns	294	100.0
T-cell prolymphocytic leukaemia	256	87.1
Adult T-cell leukaemia/lymphoma	13	4.4
Precursor T-lymphoblastic lymphoma/leukemia	7	2.4
Peripheral T-cell lymphoma, NOS	7	2.4
Sezary syndrome	4	1.4
Hepatosplenic T-cell lymphoma	4	1.4
T-cell large granular lymphocytic leukaemia	1	0.3
Angioimmunoblastic T-cell lymphoma	1	0.3
Primary cutaneous CD4 positive small/medium T-cell lymphoma	1	0.3

Conclusion

Sheffield Teaching Hospitals NHS Foundation Trust, a UKAS proficiency testing provider No. 7804, operating UK NEQAS for Leucocyte Immunophenotyping.

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Leukaemia Diagnostic Interpretation trial 151601

Case History

A 57 year old female was admitted to hospital following emergency admission through Accident and Emergency. She had severe discomfort in her upper abdomen and on physical examination was found to have both hepato-splenomegaly and lymphadenopathy. The patient died within 24 hours. Post mortem examinations of the spleen showed a dense red pulp infiltrate which had invaded the spleen capsule and had atrophied white pulp. Lymph nodes had diffuse involvement predominately in the paracortical areas.

Haematological examination revealed the following:
Hb- 96 g/L; WBC- 437.59 x10^9/L; PLT- 32 x10^9/L; RBC- 2.96x10^12/L; HCT- 0.297 L/L; MCV- 100.3 fL; MCH- 32.4 pg

Immunophenotype

The consensus results of the malignant cells had the following phenotype (consensus percentage expression in brackets).

Positive antigens – CD2 (97.65%), CD3 (98.10%), CD4 (97.02%), CD5 (98.06%), CD7 (96.92%), CD38 (40.69%), CD45 (100%), TCRab (97.48%)

Negative antigens – CD1a (0%), CD8 (1.14%), CD10 (0%), CD13 (0%), CD19 (0.3%), CD20 (0.23%), CD25 (4.79%), CD34 (0%), CD56 (0.77%), HLADR (1.11%), TCRgd (0%), TDT (0%)

Cytogenetics and Molecular genetics

45~47,XX,add(1)(q41),add(6)(q27),add(6)(q22),add(7)(q36),add(12)(p1),-13,t(13;17)(q12;q25),inv(14)(q11q32),+mar[cp15]/
46,XX[1]

Analysis showed a complex karyotype with multiple structural chromosome abnormalities including additional material on chromosomes 1 and 6 and an inversion in the long arm of chromosome 14.

FISH analysis confirmed that the inverted chromosome 14 resulted in rearrangement of the T-cell receptor α (TRA) locus.

T cell receptor (TCR) genes were clonally rearranged.

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Peripheral Blood Morphology

Morphology comments from Professor Wendy Erber
Consultant Haematologist, University of Western Australia, Nedlands, Australia

The blood film shows a marked lymphocytosis. The lymphocytes are small and round; occasional larger cells are present. They have a high nucleus: cytoplasmic ratio with irregularly nuclear contours (convolutions and indentations) and mature chromatin. Approximately 5% have a nucleolus. The cytoplasm is basophilic and the majority of cells have azurophilic granules. The red cells and neutrophils have normal morphology. Thrombocytopenia is noted.

Comment:

Mature lymphoid neoplasm. The morphology is not diagnostic. The differential diagnosis includes:

1. T Cell Prolymphocytic Leukaemia - the cytoplasmic granules are atypical for this disorder.
2. CD4-positive T cell Large Lymphocytic Leukaemia - this is a rare disorder (as the majority of cases are CD8-positive). In addition the lymphoid cells in this case are not large.
3. Adult T-cell leukaemia/lymphoma - these cases are generally CD7-negative and this small cell morphology is usually associated with the chronic form of the disease.

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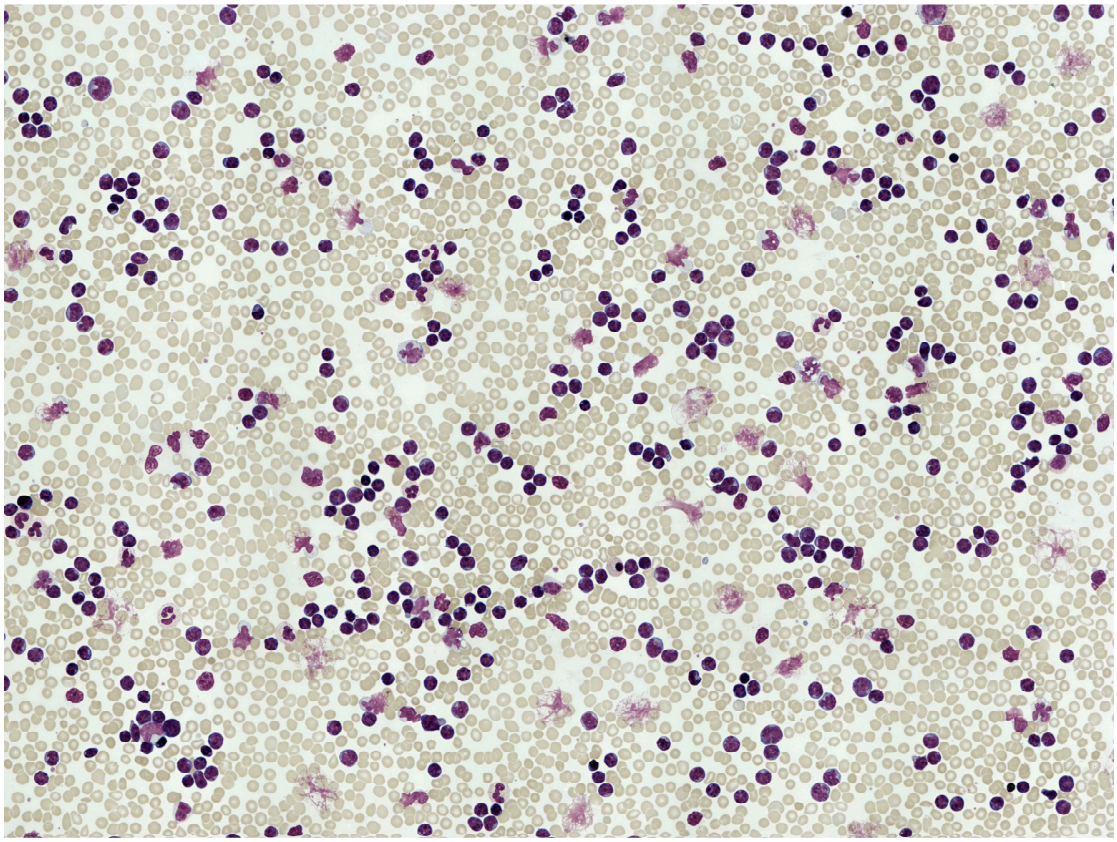
Examples of Digital Images Issued

Figure 1: Peripheral Blood x50 magnification (May Grünwald Giesma stain)



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Exercise Conclusion / Case Discussion

Please note that, because this trial requires a diagnosis based on the WHO guidelines, this report is therefore based on the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th Edition[2].

Leukaemia Immunophenotyping - Part one

On this send out there were no major issues with respect to fluorochrome variations seen.

Leukaemia Diagnostic Interpretation - Part Two

Please note that in all the cases we issue, the introduction of pathological results **by the participant** that were not provided, for example chromosome abnormalities, will mean an immediate classification of a major error. Also, all patients have had an extensive work up and thus all relevant information is provided. Where there is no evidence provided this should be regarded as being negative for this particular case.

EXERCISE CONSENSUS DIAGNOSIS (Classified as Correct)

T-cell Prolymphocytic Leukaemia

Other Differential Diagnoses (Classified as Major Errors):

Adult T-cell Leukaemia/Lymphoma
Precursor T-Lymphoblastic Lymphoma/Leukaemia
Peripheral T-cell Lymphoma, NOS
Sezary Syndrome
Hepatosplenic T-cell Lymphoma
T-cell Large Granular Lymphocytic Leukaemia
Angioimmunoblastic T-cell Lymphoma
Primary Cutaneous CD4 positive small/medium T-cell lymphoma

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Other Differential Diagnoses (Classified as Minor Errors):

No differential diagnoses were considered minor errors

The classification of major and minor errors is currently under review and will be discussed with our steering committee in the near future.

Rationale for Correct Classification

The Steering Committee considered this case to be compatible with T-cell Prolymphocytic Leukaemia because the immunophenotypic profile of the case combined with the cytogenetics supports the diagnosis although the morphology is not typical.

T-prolymphocytes are negative for TdT and CD1a. The peripheral T-cells express CD2, CD3 and CD7. The WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues 4th Edition, states that 60% cases are CD4+, CD8- as observed in this case. There was also evidence of T-cell receptor (TCR) gene rearrangement indicating clonality. There was also an inversion of chromosome 14 with breakpoints in the long arm at q11 and q32 (inv(14)(q11q32), this is observed in 80% of cases thus increasing the probability of this case being a T-cell Prolymphocytic Leukaemia. The morphology, whilst not diagnostic in this case is most suggestive of T-cell prolymphocytic leukaemia.

Rationale for Major Error Classification

The following diseases were classified by members of our Steering Committee as major errors due to incompatible phenotypes, morphology or associated cytogenetics. The following disease categories would also have had potential treatment implications.

Adult T-cell leukaemia/lymphoma: The phenotype is not consistent with this disease. Phenotypically, ATLL cases usually lack CD7 and CD25 is strongly expressed in nearly all cases. This case was positive for CD7 and CD25 negative. Furthermore, whilst morphologically this has to be considered as a possible differential diagnosis, no poly-lobated ‘flower-cells’ were observed although it is acknowledged that the small cell morphology observed in this case can be associated with the chronic form of the disease, the cytogenetics and immunophenotype mean that this disease entity should be excluded. Furthermore, ATLL is highly associated with the human T-cell leukaemia virus type 1 (HTLV-1) and as stated above because this was not provided this should be regarded as being negative.

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Precursor T-lymphoblastic lymphoma/leukaemia: Cells in this condition are typically medium sized but where small lymphoblasts are present they have no evident nucleoli. Morphology comments for this case stated that approximately 5% of the lymphocytes exhibited nucleoli. However, this case was also phenotypically inconsistent with T-ALL/LBL. Such cases usually express TdT and variably express CD1a, both of which were negative in this case. Furthermore, CD34 was negative in this case; this coupled with the genetics means that this diagnosis can be excluded.

Peripheral T-cell lymphoma, NOS: Whilst this category of disease comprises of approximately 30% of cases in the Western world the white cell count in this case was extremely high and such peripheral blood leukaemic presentation in PTCL, NOS cases is highly unusual. Again, whilst morphologically this case should be included as a possible differential diagnosis once the pathologies are included this disease category can be excluded. Whilst it is accepted that cytogenetic abnormalities can be complex this condition is characterised by aberrant T-cell phenotype with down regulation of CD5 and CD7. Both of these markers were positive in this case.

Sezary Syndrome: The morphology and immunophenotype are inconsistent with this disease. No Sezary cells (T-cells with cerebriform nuclei) were in evidence. Additionally, Sezary cells characteristically lack CD7.

Hepatosplenic T-cell Lymphoma: Inconsistent immunophenotype and cytogenetics. In this condition, neoplastic cells are usually negative for TCRαβ, CD4 and CD5. All of these markers were positive in this case. Inversion of chromosome 14 is not characteristic of this disease

T-cell Large Granular Lymphocytic Leukaemia: The morphology was not considered to form part of a differential diagnosis. This condition is characterised by the presence of large granular lymphocytes. The morphology comments for this case stated that the lymphocytes were small and round. Furthermore, there was inconsistent presentation. The condition is typically indolent and non progressive and the total white cell count would not normally reach the level observed in this case. No unique cytogenetic abnormalities are associated with such a disease entity.

Angioimmunoblastic T-cell Lymphoma: The phenotype was inconsistent with this disease entity. This condition characteristically expresses CD10 and numerous CD8+ cells usually present, both of which were negative in this case

Primary Cutaneous CD4 Positive Small/Medium T-cell lymphoma: The presentation and high white cell count are inconsistent with this disease category. This is generally asymptomatic cutaneous condition.

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Further Trial Findings

Table 1: Submissions from all participants - laboratories and individuals.

Diagnosis	Total No. of Returns	No. of Laboratories	No. of Individual
T-cell Prolymphocytic Leukaemia	256	179	77
Adult T-cell Leukaemia/Lymphoma	13	6	7
Precursor T-Lymphoblastic Lymphoma/Leukaemia	7	4	3
Peripheral T-cell Lymphoma, NOS	7	7	0
Sezary Syndrome	4	2	2
Hepatosplenic T-cell Lymphoma	4	2	2
T-cell Large Granular Lymphocytic Leukaemia	1	0	1
Angioimmunoblastic T-cell Lymphoma	1	1	0
Primary Cutaneous CD4 positive small/medium T-cell lymphoma	1	1	0

Table 2: Submissions from laboratory participants only

Diagnosis	Laboratory Returns %
T-cell Prolymphocytic Leukaemia	88.6
Adult T-cell Leukaemia/Lymphoma	3.0
Precursor T-Lymphoblastic Lymphoma/Leukaemia	2.0
Peripheral T-cell Lymphoma, NOS	3.5
Sezary Syndrome	1.0
Hepatosplenic T-cell Lymphoma	1.0
T-cell Large Granular Lymphocytic Leukaemia	0
Angioimmunoblastic T-cell Lymphoma	0.5
Primary Cutaneous CD4 positive small/medium T-cell lymphoma	0.5

Table 3: Submissions from individual participants only

Diagnosis	Individual Returns %
T-cell Prolymphocytic Leukaemia	83.7
Adult T-cell Leukaemia/Lymphoma	7.6
Precursor T-Lymphoblastic Lymphoma/Leukaemia	3.3
Peripheral T-cell Lymphoma, NOS	0
Sezary Syndrome	2
Hepatosplenic T-cell Lymphoma	2
T-cell Large Granular Lymphocytic Leukaemia	1
Angioimmunoblastic T-cell Lymphoma	0
Primary Cutaneous CD4 positive small/medium T-cell lymphoma	0

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References:

1. Bain BJ, Barnett D, Linch D, Matutes E, Reilly JT. Revised guidelines on immunophenotyping in acute leukaemias and chronic lymphoproliferative disorders. Clin.Lab. Haem. 2002;24:1-13

2. Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th Edition. IARC Press 2008

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Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
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e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance. Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided.

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